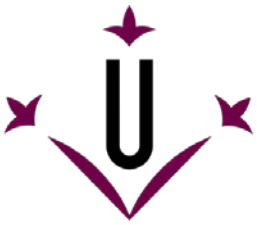

Modeling the Inactivation of *Listeria innocua* and *Escherichia coli* in Fresh-Cut Tomato Treated with Pulsed Light

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Abstract

The effectiveness of pulsed light (PL) treatments to inhibit microorganisms on fresh-cut tomatoes (*Lycopersicon esculentum* Mill., cv. Daniela) was investigated. Tomato slices inoculated with *Escherichia coli* or *Listeria innocua* were exposed to PL-treatments (4, 6 or 8 J cm^{-2} fluence) and kept cold at 4 ºC for 20 days. *L. innocua* and *E. coli* counts, gases in the headspace of the containers (O_2 and CO_2), pH, titratable acidity and soluble solids content were monitored throughout the cold storage. The PL-treatments reduced significantly ($p< 0.05$) initial loads of both microbes. The effect of the PL-fluence on the survival number of microoganisms was described by a log-linear model ($R^2= 0.849$-0.999). At any fixed time within the cold storing, the microbial counts for untreated samples were always higher than those cut tomatoes that had been previously PL-treated. The behaviour of *L. innocua* and *E. coli* during the storage were well adjusted ($R^2> 0.930$) by Gompertzian models; the studied microorganisms exhibited different patterns during the storage period. On the other hand, O_2 and CO_2 partial pressures in containers with fresh-cut tomatoes were also significantly affected by PL-treatments ($p< 0.05$). The highest PL-fluence caused the greatest changes of O_2 and CO_2 contents. In addition, the application of PL triggered an acceleration of the O_2 consumption during the cold stage. PL-treatments might be used to effectively extend the safety of fresh-cut tomatoes over 12 days of storage against *E. coli* and *L. innocua* growth.

Keywords: fresh-cut tomato; pulsed light treatments; *Listeria innocua*; *Escherichia coli*; headspace gases

1. Introduction

Tomato is one of the most widely consumed vegetables either fresh or processed. Consumption of tomatoes is now considered as an indicator of good nutritional habits and healthy lifestyle mainly because of the presence of vitamins, phenolic compounds, flavonoids and, especially, carotenoids such as lycopene and β-carotene in the product (Odriozola-Serrano et al. 2008).

The growing demand from consumers for healthy, convenient and fresh-like products has motivated to study the effects of minimum processing on the quality of fruits and vegetables. Mechanical operations such as slicing, shredding or dicing bring about a rapid deterioration of vegetables involving physical and chemical changes (Francis and O'Beirne 2005). Moreover, fresh-cut fruits and vegetables are even more susceptible to spoilage due to the release of nutrient and cellular fluids by disruption of protective epidermal layers (Siddiqui et al. 2011). Indeed, fresh foods consumption remains as a major cause of outbreaks of foodborne diseases (Newell et al. 2010).

On the other hand, pathogenic microorganisms may contaminate the products and, thus, the risk of foodborne diseases increases (Beuchat 1996a). Foodborne pathogens, such as *Listeria monocytogenes* and *Escherichia coli*, may also be present on plant foods; a number of outbreaks associated with consumption of different vegetables contaminated with *L. monocytogenes* (Francis et al. 1999; Sagoo et al. 2003) and *E. coli* O157:H7 (Ethelberg et al. 2010; Friesema et al. 2008) have been reported. In fact, the occurrence of these microorganisms has been observed on the surface of fresh-cut tomatoes (Asplund and Nurmi 1991; Beuchat 1996b).

Nowadays, researchers have focused on the study of non-thermal technologies for microbial inactivation to minimize the negative impact on the physicochemical, nutritional and sensorial characteristics of fresh-cut products. Among these preservation treatments, pulsed light (PL) is emerging as an alternative to the disinfection of fruits and vegetables surfaces. This technology involves the application of light pulses using intense broad spectrum light for short periods and it is able to inactivate vegetative bacteria, spores, yeast and molds (Oms-Oliu et al.

2010). The germicidal effect of the PL-treatments has been mainly attributed to photochemical or photothermal actions (Gómez-López et al. 2005), as well as to the physicochemical composition of fresh-cut commodities (Ramos-Villarroel et al. 2012). Likewise, an important part of the microbial studies is the search of mathematical models with the aim of predicting microbial growth or depletion considering the process conditions and the storage time (Palacios et al. 2014).

Several studies have shown the ability of PL to inactivate spoilage and pathogenic microorganisms in processed and minimally processed fruits and vegetables using *L. innocua* and *E. coli* as surrogate strains for *L. monocytogenes* (Ramos-Villarroel et al. 2014) and *E. coli* O157:H7 (Palgan et al. 2011), respectively. Therefore, the choice of adequate surrogate strains in studies for validation of processes can avoid the difficulties (mainly, pathogenicity) that the target microorganisms could have (FDA/CFSAN 2001).

Hence, the objectives of the present work were to evaluate the impact of different PL-treatments on the growth of surrogate-pathogenic strains, *Escherichia coli* and *Listeria innocua* bacteria, inoculated on fresh-cut tomato as well as to model the changes on the microbial growth and the oxygen and carbon dioxide partial pressures in the package headspace throughout cold storage.

2. Materials and Methods

2.1 Raw materials and processing

Tomatoes (*Licopersicon esculentum* Mill. cv. Daniela) were purchased in a local supermaket (Lleida, Spain) at red stage, characterized by red color in more than 90% of the surface, as defined by the U.S. standards for grades of fresh tomatoes (CFR, 1991) and kept under refrigerated conditions (4 ºC) before use.

Fresh whole tomatoes were sanitized by immersion in chlorinated water (100 ppm) at 4 ºC for 2 min, rinsed with tap water and gently dried by hand. Tomato fruits were then cut into 5 mm-thick slices using an electric slicer (Food Slicer-6128: Toatmaster Corp, Elgin, U.S.A.).

93 Batches of three tomato slices (50 g) were placed in polypropylene trays and immediately
94 inoculated with strains of *Listeria innocua* 1.17 and *Escherichia coli* 1.107.

95 2.2.1 Preparation of inocula

96 *Listeria innocua* CLIP11262 isolated from cheese and *Escherichia coli* 1.107 isolated
97 from human feces were used as surrogates for the pathogenic bacteria *L. monocytogenes* and *E.*
98 *coli* O157:H7, respectively. The original strains were kept on tryptone soy agar, (TSA: Biokar
99 Diagnostic; Beauvais, France) into inclined test tubes at 5 ºC until use. A stock culture from *L.*
100 *innocua* was grown in tryptone soy broth (TSB) with 0.6% (w/v) yeast extract (Biokar
101 Diagnostics; Beauvais, France) at 35 ºC for 15 h at 100 rpm, whereas a stock culture of *E. coli*
102 was grown in TSB (Biokar Diagnostics; Beauvais, France) at 37 ºC during 11 h at 80 rpm.
103 These conditions of incubation were performed to obtain cells near their stationary growth
104 phase (10^8 CFU ml^{-1}). Then, the cells in the stock cultures were resuspended using saline
105 peptone water (Sharlau Chemie, S. A.; Barcelona, Spain) in agar and broth for dilution assays,
106 respectively, up to cell densities of approximately 10^6 CFU ml^{-1}, which were subsequently used
107 as inoculum cultures.

108 2.2.2 Inoculation of tomato slices and packaging

109 Fresh-cut tomatoes (50 g) were inoculated by spreading 500 µL of *L. innocua* or *E. coli*
110 stocks cultures (10^6 CFU ml^{-1}) over their entire surface with a sterile micropipette and extended
111 with a sterile Digralsky spreader. Immediately after the inoculation, transparent polypropylene
112 trays (350 cm^3, 5025 RM PTT-ATS Packaging S.r.l.; Venice, Italy) were filled up with the
113 tomato slices and the trays were then thermally sealed with plastic film using an ILPRA Food
114 Pack Basic V/6 packaging machine (ILPRA Systems, CP; Vigevono, Italy). According to
115 previous studies (Avalos-Llano et al, 2016), the transparency of the film was 97% of the UV-
116 radiation and almost a 100% of the visible wavelengths. Furthermore 85% of the energy
117 associated to the 200-320 nm range reached the surface of the samples. The permeabilities for
118 oxygen and carbon dioxide through the transparent sealing film were 5.2419 x 10^{-13} mol O_2 m^{-2}

119 s^{-1} Pa^{-1} and 2.3825 x 10^{-12} mol CO_2 m^{-2} s^{-1} Pa^{-1} at 23 ºC and 0% relative humidity, respectively
120 (ILPRA Systems España, S.L.; Mataró, Spain). After the sealing, the trays with the slices of
121 inoculated tomato were immediately subjected to PL-treatments.

122 2.3 Pulsed light treatments

123 PL-treatments were carried out using an automatic laboratory flash lamp system
124 (Steribeam Xe-Matic-2L-A; Kehl, Germany). The emission spectrum of the light source ranged
125 from 200 to 1100 nm. The duration of each pulse was 0.3 ms with 0.4 J cm^{-2} fluence per
126 emitted pulse from two xenon lamps at 8.5 cm above and below the sample holder. To evaluate
127 the effect of applying different treatment doses, inoculated samples were subjected to 10, 15 or
128 20 pulses. Hence, the applied fluences per each side were 4, 6 and 8 J cm^{-2}, respectively.
129 Treatment intensities were selected on the basis of pre-trials among those not causing
130 undesirable sensory changes to the product. Transparency of the film was determined by
131 measuring the amount of energy received by a photodiode detector placed at the sample holder
132 (Avalos-Llano et al., 2016). Energy calculations were carried out according to the calibration
133 with a standard light source following the manufacturer's directions. A set of untreated samples
134 was kept as control reference. To observe the initial effects of PL processing, a number of
135 determinations were carried out just after sealing and PL processing (about 30 min). Finally,
136 sample trays were stored at 4 °C for 20 days in darkness until random withdrawal for analysis.

137 2.4 Headspace gases analysis

138 The atmosphere of each package was analyzed using a gas chromatograph equipped with
139 a thermal conductivity detector (Micro-GP CP 2002 gas analyzer; Chrompack International;
140 Meddelburg, Netherlands), so a sample of 1.7 ml was automatically withdrawn from the
141 headspace atmosphere and fed in the chromatograph. Portions of 0.25 and 0.33 ml were injected
142 for O_2 and CO_2 determinations, respectively. A CP-Molsieve 5 Å packed column (4 m x 0.32
143 mm, d.f.= 10 mm) at 60 ºC and 100 kPa was used to determine the O_2 partial pressure; on the
144 other side, to determine the CO_2 partial pressure, a Pora-PLOT Q column (10 m x 0.32 mm,
145 d.f.= 10 mm) held at 70 ºC and 200 kPa was used (both columns by Chrompack International;

146 Middelburg, Netherlands).

147 2.5 *Listeria innocua* and *Escherichia coli* counts

148 Tomato slices (10 g) were aseptically removed from each tray and transferred into sterile
149 stomacher bags (Standard bags, circulator 400, Stomacher®; Sussex, United Kingdom)
150 containing 90 ml of 0.1% (w/v) saline peptone water (Biokar Diagnostics, Beauvais, France)
151 and 0.85% (w/v) of NaCl (Sharlau Chemie, S. A.; Barcelona, Spain) and homogenized for 3
152 min in a stomacher blender (IUL Instruments; Barcelona, Spain). *E. coli* and *L. innocua* counts
153 were carried out by direct plating technique. Serial dilutions were made and poured at reason of
154 0.1 ml on MacConkey and Palcam agar-selective supplement (Biokar Diagnostic; Beauvais,
155 France) plates in duplicate for *E. coli* and *L. innocua* counts, respectively. Plates were incubated
156 for 24-48 h at 35-37 ºC. A 50971-Colony Counter (Bioblock Scientific; Taiwan) was used for
157 enumeration of colonies and the results were expressed as Log ($CFUg^{-1}$).

158 2.6 Soluble solids, pH and acidity determinations

159 On each sampling day, inoculated tomato slices (20 g) from each tray were homogenized
160 in a blender. The juice was then filtered through a 2-mm diameter steel sieve to remove peel and
161 seeds. An aliquot was used to determine total soluble solids (TTS) using a 2WAJ-ABBE
162 Refractometer (Atago Company Ltd.; Tokyo, Japan) and expressed as Brix degrees (ºBx). The
163 pH was measured using a pH-meter (Crison Instruments S. A.; Barcelona, Spain). Total acidity
164 (TA) was assessed by titration with NaOH (0.1 N) to pH 8.1 and the results were expressed as
165 grams of anhydrous citric acid per 100 g of fruit.

166 2.7 Statistical analysis and mathematical modeling

167 Each processing condition was assayed in duplicate at each sampling time and three
168 replicate analyses were carried out for each trail (every 4 days throughout 20 days storage) to
169 obtain the mean value (n= 6). Significance of the results and statistical differences were
170 analyzed using the Statgraphics plus v. 5.1 Windows package (Statistical graphics Co.;
171 Rockville, MD, U.S.A.). Analysis of variance (ANOVA) was performed to compare sample

mean values. Also non-linear regression procedures were used to fit the experimental data to the models. LSD multiple range tests were applied to determine differences among means. The significance level for statistical tests and confidence intervals for all estimated parameters was p= 0.05.

Log-linear relationships, which are expressed by Equation 1, were used to describe the effect of PL-treatments on the microorganisms before the cold storage of the trays of the tomato slices. In the Equation 1, N_0 is the initial count of the microorganisms (CFU g^{-1}); N denotes the number of survival microorganisms after different PL-fluences Φ (J cm^{-2}); and δ (cm^2 J^{-1}) is an inactivation constant that depends on the microorganism and the rest of experimental conditions.

$$Log\, N = Log\, N_0 - \delta \cdot \Phi \quad (1)$$

On the other hand, second order polynomials, Equation 2, were used to relate the partial pressures of oxygen (C_o) or carbon dioxide (C_{CO2}) in the headspace of the trays as a function of the applied fluence (Φ) throughout each PL-treatment. In this equation, C_G denotes the partial pressure (kPa) of either oxygen or carbon dioxide and a, b and c are the parameters of the mathematical model.

$$C_G = a\Phi^2 + b\Phi + c \quad (2)$$

A Gompertz´s model, Equation 3, was used to describe the changes on the microbial growth as well as the in-packages O_2 and CO_2 partial pressures as a function of the storage time. (Flores-Cervantes, et al 2013; Oms-Oliu et al., 2007; Lancioti et al., 1999).

$$y = A + C\, exp\left\{-exp\left[-B\left(t - M\right)\right]\right\} \quad (3)$$

In equation 3, y denotes the decimal logarithm of the microorganism counts [Log (CFU g^{-1})] or, also, the oxygen or carbon dioxide partial pressures (kPa); A are the values of the anterior variable (y) just at the beginning of their storage period; depending on y meaning, A value was linked to the outputs given for this parameter by prior models (Equation 1 or Equation 2); B is the relative rate of change at time equal to M; M is the time (day) at which the

198 absolute rates of change is maxima; *C* is the difference between the initial and final asymptotic
199 values attained for each variable during the cold storage; and *t* is the storage time.

200 From the values of Gompertz´s model parameters, the maximum exponential rates of
201 change (μ_{max}) and the time before the beginning of changes, lag time (λ), were calculated using
202 the Equation 4 and Equation 5 in which $A_{e,}$ are the experimental values of the considered
203 variable at the beginning of the storage period; and *e* is the Euler's number.

204 $$\mu_{\max} = \frac{B \times C}{e} \quad (4)$$

205 $$\lambda = M - \left(\frac{1}{B}\right) + \frac{A_e - A}{\mu_{\max}} \quad (5)$$

206

207 **3. Results and discussion**

208 3.1 Headspace gases

209 Partial pressures of O_2 and CO_2 inside the packages of the tomato slices were significantly
210 affected by PL processing (Figure 1). The oxygen partial pressure inside the packages (20.9
211 kPa) at the beginning of the cold storage decreased immediately after exposure to PL; the
212 maximum depletion (up to 17.4 kPa O_2) took place at the highest applied PL-fluence (8 J cm^{-2}).
213 Conversely, carbon dioxide partial pressure (0.2 kPa CO_2) at the start of this period of time
214 increased significantly ($p < 0.05$) after the exposure to PL-treatments; indeed, CO_2 pressure
215 reached 0.91 kPa at 8 J cm^{-2}. The fit of second-order polynomial functions to O_2 or CO_2
216 concentration as a function of the fluence of the PL-treatments led to Equation 6 and Equation
217 7, respectively.

218 $$C_{O_2} = -0.0048\Phi^2 + 0.123\Phi + 0.209 \quad (R^2 = 0.967)$$

219 $$C_{CO_2} = 0.0161\Phi^2 - 0.627\Phi + 20.8 \quad (R^2 = 0.998)$$

220 These mathematical models point that PL-treatments bring on a rapid consumption of O_2
221 that triggers an accelerated production of CO_2 from the tomato slices. To date, no report on the
222 effect of PL on the respiration of tomato slices is known. However, the pattern observed for the

223 headspace composition in the trays with tomato slices was in agreement with the one reported
224 by Ramos-Villarroel et al. (2011a) who found that O_2 and CO_2 contents on PL-treated avocado
225 slices were more affected when higher PL-fluences were applied. In this context, Gómez-López
226 et al. (2005) reported that PL-treatments affect the plant cells causing DNA damages, disorders
227 in tissues and photosynthetic apparatus and, thus altering the respiration of the vegetables.
228 During the cold storage period, oxygen and carbon dioxide partial pressures inside the
229 packages decreased and increased, respectively, at any applied PL-fluence. Figure 2 and Figure
230 3 show that the higher applied PL-fluence the higher effect on the changes of partial pressures
231 of O_2 and CO_2 during the cold storage. Indeed, a PL-fluence of 8 J cm^{-2} yielded lower O_2 and
232 higher CO_2 contents (0.12 and 16.60 kPa, respectively) compared to untreated samples (0.91
233 and 17.40 kPa, respectively) at the end of the storage.
234 Both the progress of O_2 and CO_2 partial pressures during the storage period were
235 described by the Gompertz´s model (Equation 3). The estimates of the parameters of these
236 models, determination coefficients (R^2) as well as other derived quantities are given in Table 1.
237 The values of the specific consumption rate of O_2 (μ_{max}) were negative for both PL-treated and
238 untreated slices of tomato; this indicates that the prior processing had yet triggered the
239 accelerated consumption of oxygen during the storage. Moreover, the highest lag time value (λ)
240 was obtained for the assay without PL-treatment. In contrast, lag phase values were shorter as
241 the PL-fluence increased, as a quicker depletion in oxygen levels occurred.
242 With regard to the carbon dioxide production, the assayed model fitted the experimental
243 data with good accuracy (R^2= 0.994-0.973). The kinetic parameters μ_{max} (0.39-0.97 kPa^2 day^{-1})
244 and λ (< 1.65 day) values indicate an accelerated CO_2 production triggered after about 2 days
245 and the greater the applied PL-fluence, the greater the production rate of CO_2 during the cold
246 storage. Increases of the CO_2 rates after cutting of fresh tomatoes have been previously reported
247 (Ahmed et al. 2012; Gil et al. 2002); these changes were related to an acceleration of respiration
248 due to physical stress caused by processing. Indeed, mechanical processing (slicing) as well as
249 PL-treatment could cause an initial damage of the fruit tissue, triggering an accelerated
250 respiration of fresh-cut tomatoes through storage. Moreover, Ramos-Villarroel et al. (2014)
251 indicated that changes on the headspace composition through storage could be well related to

252 accelerated respiration caused by modifications of the products physiology due to initial PL
253 treatments.

254 3.2 Microbial inactivation

255 The *Listeria innocua* counts on tomato slices decreased significantly ($p< 0.05$) just after
256 exposure to PL-treatments. The higher applied PL-fluence (Φ) the higher reduction of the *L.*
257 *innocua* counts (Figure 4). The microbial inactivation obtained just after exposition to PL as a
258 function of Φ could be described using a log-linear equation (R^2= 0.849) in which the
259 inactivation constant was δ= 0.122 $cm^2 J^{-1}$. Thus, the *L. innocua* counts (N) estimated by the
260 log-linear model (Equation 1) before cold storage were 6.17, 5.76, 5.54 and 5.27 [Log (CFU g^{-}
261 ^{1})] for the PL-untreated tomato slices and for those submitted to 4, 6 and 8 J/cm^2, respectively.
262 Uesugi et al. (2013) could reduce up to 4-5 Log units *L. innocua* in liquid substrate after
263 exposure to a 5 J cm^{-2} PL-treatment. This higher microbial reduction might be due to the
264 differences on the matrix where the bacteria were inoculated in. Indeed, physicochemical
265 factors such as chemical composition, total soluble compounds, pH and light absorbance
266 (specially due to compounds as carotenoids) could potentially protect the microorganisms from
267 the PL-treatments and, thus, a different inactivation of the microorganisms is achieved.
268 During the cold storage, the counts of *L. innocua* on fresh-cut tomatoes that were
269 subjected to PL-treatments did not significantly change through the first 12 days of storage,
270 while progressive increases of *L. innocua* counts on the untreated tomato slices were observed
271 after 4 days (Figure 5). Regardless the PL-treatment that was applied to the tomato slices, the *L.*
272 *innocua* loads increased from day 12 to day 16 reaching a plateau by day 20. However, at day
273 12 differences in the microbial counts were evident. The lowest ($p<0.05$) *L. innocua* counts (5.9
274 Log CFU g^{-1}) were found on fresh-cut tomatoes exposed to a fluence of 8 J cm^{-2} while the
275 highest corresponded to the untreated samples (8.4 Log CFU g^{-1}).
276 The modified Gomperz´s model provided a good fit to the experimental data ($R^2> 0.930$).
277 As can be seen in Table 2, the differences among the C values for *Listeria innocua* were not
278 enough significant to assess the influence of PL-fluence on the microbiological counts. It is

noticeable to point out that the lag phase period (λ), which corresponds to the period when de microorganism is adapting itself to the medium conditions and is unable to divide, became visibly lengthened when PL-treatments were applied; indeed, for untreated tomato slices, λ was 4.25 day, meanwhile, for PL-treated samples, λ ranged from 8.06 to 9.48 day. Moreover, this significant ($p< 0.05$) increment of λ is coupled with the fact that the higher fluence applied to samples, the higher values for time at which maximum growth rate (M) is reached (6.95 day and from 10.87 to 12.22 day for untreated and PL-treated tomato slices, respectively); besides, the relative growth rate (B) decreased from 0.49 to 0.24 Log (CFU·g^{-1})·day^{-1} as PL-fluence increased from 4 to 8 J cm^{-2}; likewise, maximum exponential growth (μ_{max}) decreased from 0.33 to 0.22 Log (CFU·g^{-1})·day^{-1} as PL-fluence increased. Thus, *L. innocua* exhibited a higher resistance to PL treatments but, at the same time, the growth is delayed throughout the storage period with respect to untreated fresh-cut tomatoes.

Regarding *Escherichia coli* counts, significant decreases ($p< 0.05$) of about 0.8 and 1.1 Log (CFU g^{-1}) were observed on fresh-cut tomato slices immediately after its exposure to 4 and 6 J cm^{-2} PL-fluence (Figure 4), respectively. Further reduction of initial load of *E. coli*, 1.4 Log (CFU g^{-1}), was achieved applying 8 J cm^{-2} PL-fluence on the slices. The fit of a log-linear model (Equation 1) to the observed depletion of the microbial load as a function of the fluence (Φ) yielded a high agreement (R^2= 0.999) between the experimental data and the values predicted by the model. In this case, the inactivation constant was δ= 0.172 cm^2 J^{-1}, which was a clear sign of less resistance to PL-treatments by *E. coli* in comparison with *L. innocua*. Hence, the decimal logarithm of the microorganism counts obtained from Equation 1 [6.34, 5.28, 5.18 and 4.72 Log (CFU g^{-1})] for tomato slices untreated or exposed to 4, 6 and 8 J cm^{-2} fluence, respectively, was the estimated value for the microbial load on the tomato slices just at the beginning of their cold storage stage (parameter *A* in Equation 2).

Conversely to behavior of the *L. innocua* on the tomato slices during the cold storage, a significant inactivation of *E. coli* was observed during this period (Figure 6). Moreover, at a fixed cold storage stage, the number of microorganisms in the fresh-cut tomatoes that had been subjected to PL-treatments was lower than in the untreated cut tomatoes, whichever of the PL-

307 fluence applied to. So at the end of the cold stage, *E. coli* count on untreated fresh-cut tomato
308 slices was 3.8 Log (CFU g^{-1}) whereas for the slices that had been exposed to PL fluences of 4, 6
309 and 8 J cm^{-2}, the final counts were 2.8, 2.7 and 2.1. Log (CFU g^{-1}), respectively. As was
310 likewise reported for *L. innocua*, Gompertzian models fitted accurately the experimental data
311 obtained for the decline in the counts of *E.* coli on the tomato slices as a function of the cold
312 storage time (Figure 6); the determination coefficients (R^2), which ranged from 0.930 to 0.997,
313 and the parameters of the model at the assayed PL-fluences are listed in Table 2. The highest
314 values for B and μ_{max} parameters corresponded to the untreated cut tomatoes, while those ones
315 that were exposed to PL-treatments exhibited lesser values for these parameters; this fact
316 indicates that both relative death rates and maximum rates of death for *E. coli* were significantly
317 ($p< 0.05$) lower for the PL-untreated tomato slices, which might be related to the lower initial
318 microbial loads that the PL-processing had previously caused. However, both the time at which
319 death rates were maxima (M) and the lag time (λ), in which no growth of the microorganisms
320 occurs; were significantly ($p< 0.05$) shortened when PL-treatments were applied regardless the
321 fluence; these results pointed out the inactivation of *E. coli* become accelerated after PL-
322 treatments and suggest that the microorganism was highly susceptible to them. Therefore,
323 tomato slices exposed to higher fluences yielded lower microbial counts for a longer time than
324 those untreated ones and, thus extending the safety of fresh-cut tomatoes up to 12 days after
325 processing. In this respect, some studies have reported that *E. coli* was inactivated in solid and
326 liquid substrates and fresh-cut products after exposure to similar PL-fluences and storage
327 conditions (Uesugi et al. 2013; Kramer and Muranyi, 2014; Ramos-Villarroel et al. 2011a;
328 Ramos-Villarroel et al. 2011b).

329 As it has been showed, a clear influence of PL-treatments on the evolution of *L. innocua*
330 and *E. coli* during the cold storage stage was observed. Ramos-Villarroel et al. (2012) suggested
331 that the variation in the reductions of *L. innocua* and *E. coli* counts on fresh-cut watermelon
332 subjected to PL-treatments can be well explained by differences in the cell wall composition
333 and structure of these microorganisms. Indeed, *E. coli* is a Gram-negative bacterium with less
334 rigid and thinner cell wall than *L. innocua*, which is a Gram-positive one. Some authors (Rowan
335 et al. 1999; Anderson et al. 2000) have demonstrated that Gram-negative bacteria are more

susceptible to PL-treatments due to the environment where they grow. Sensibility of *Listeria* and *Escherichia* strains to PL-treatments is dependent of the environmental conditions and decontamination treatment applied (Rajkovic et al. 2010). In this context, Gram-negative bacteria are enteropathogens restricted to darkness in humans and animals tracts and do not develop resistance to the presence of light; on the other hand, Gram-positive bacteria can be found in all types of environments and, hence, they are more light exposed, what made them more resistant to light damages (Hobbs and Robert 1987).

The different pattern of behavior observed for the microorganisms studied in present work throughout the storage period, either with or without previous PL-processing, were also determined by the storage temperature and other environmental factors intrinsic to the product. Ramos-Villarroel et al. (2012) indicated that storage temperature could also impact on the microbial growth on fresh-cut vegetables. It has been demonstrated that *E. coli* is a mesophyll that can survive at temperatures between 5 and 45 °C. On the other hand, *L. innocua* belongs to the group of psychrophiles, which are able to grow between -15 and 20 ºC. Moreover, some inherent factors of tomatoes such as acidity and pH could influence the behavior observed on *L. innocua* and *E. coli* counts through the storage period. *E. coli* generally grows within the pH range of 4.4-9.0, while *L. innocua* grows at pH of 4.0-9.6 (Riemann and Cliver 2006).

3.3 Soluble solids content (SSC), pH and titratable acidity (TA)

Table 3 shows the values of SSC, pH and TA of untreated and PL-treated fresh-cut tomato throughout the 20 days of chilled storage (4 ºC). Although SSC, pH and TA of fresh-cut tomatoes did not suffer changes just after PL-processing, significant differences ($p< 0.05$) with respect to the initial values were observed over storage. Regarding SSC, fresh-cut tomatoes exposed to PL exhibited lower values (< 4.83 ºBx) with respect to untreated samples (5.08 ºBx) at the end of storage. The slight differences in SSC on the tomato slices during the storage stage might be related to mild increases in transpiration rates, which had been triggered by unavoidable tissue breakdowns during the processing of the tomatoes. Actually, slight water exudates were observed visually in both untreated and PL-treated cut tomatoes through the cooling stage.

Although changes on pH were observed in all samples throughout chilled storage, pH values of fresh-cut tomatoes exposed to PL were lower than those on untreated samples. Furthermore, slight decreases on the acidity values of untreated and PL-treated samples were observed during the storage period. Similarly, Ramos-Villarroel et al. (2011b) reported decreased pH and acidity on PL-treated avocado attributing this effect to *L. innocua* and *E. coli* inactivation by PL-treatments. In fact, microbial deterioration, as well as physiological activity of fresh-cut tomato, play an important role in the degradation of organic acids and, in turn, affecting the pH (Odriozola-Serrano et al. 2008; Gil et al. 2006).

4. Conclusions

PL-treatment could be a good alternative to reduce the growth of pathogenic microorganisms, thus improving safety of fresh-cut tomatoes. The effectiveness of the PL-treatments to reduce the microbial counts depends of the fluence applied and the sort of the microorganism itself. It has been proved that the higher PL-fluence applied, the higher achieved inactivation of the studied microbes. *L. innocua* exhibited a greater resistance to PL-treatments compared to *E. coli*. Indeed, the patterns that the microorganisms showed during the cold stage differed significantly. The behavior of both microbes on fresh-cut tomatoes slices during cold storing was adequately described by Gompertzian models regardless of PL-treatments. Moreover, this modeling was also a useful tool for describing the O_2 and CO_2 changes in the packages headspace. It is outstanding that the O_2 consumption was slightly accelerated by PL-treatments, which showed to trigger a rapid decrease of the O_2 concentrations during the storage time. The results of the present study may contribute to the advancement of predictive models involving PL-treatments and microorganisms.

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Table 1.

Gas	PL-fluence (J cm^{-2})	*A*	*C*	*B*	*M*	μ_{max}	λ	R^2
O_2	0 (untreated)	20.9±2.65	-26.78±2.67	0.13±0.01	10.94±0.86	-1.30±0.04	3.29±0.17	0.996
	4	19.3±2.23	-21.35±2.33	0.15±0.02	8.35±0.92	-1.16±0.07	0.98±0.21	0.988
	6	18.8±7.02	-27.09±6.85	0.11±0.03	10.47±2.9	-1.06±0.04	-0.02±0.18	0.975
	8	17.4±1.05	-16.54±1.22	0.30±0.08	2.46±0.6	-1.85±0.11	-1.1±0.49	0.936
CO_2	0 (untreated)	0.21±0.02	5.28±0.25	0.20±0.03	6.50±0.41	0.39±0.06	1.3±0.28	0.994
	4	0.69±0.07	21.40±0.02	0.07±0.05	16.03±1.23	0.60±0.10	1.72±0.23	0.977
	6	0.71±0.05	19.26±0.69	0.09±0.04	10.43±4.25	0.69±0.02	-1.59±0.21	0.973
	8	0.91±0.12	17.63±2.57	0.15±0.04	7.44±1.32	0.97±0.06	1.65±0.27	0.978

A: content of the gases at the beginning of the storage (kPa); ***B***: relative rate of change at time equal to M (kPa day^{-1}); **C**: difference between the final and initial asymptotic values for the gas content (kPa); ***M***: time at the maximum rate of change (day); μ_{max}: specific change rate of O_2 consumption or CO_2 production over time (kPa day^{-1}); λ: lag time (day). R^2: determination coefficient adjusted for degree or freedom, dimensionless. Values ± SD.

Table 2.

Inoculum	PL-fluence (J cm^{-2})	*A*	*C*	*B*	*M*	μ_{max}	λ	R^2
L. innocua	0 (Untreated)	6.17±0.21	2.23±0.15	0.37±0.09	6.95±0.48	0.30±0.02	4.25±0.61	0.98
	4	5.76±0.35	1.84±0.10	0.49±0.09	10.87±0.28	0.33±0.04	8.84±0.73	0.987
	6	5.54±0.21	2.04±0.32	0.38±0.16	12.11±0.75	0.28±0.01	9.48±0.77	0.938
	8	5.27±0.28	2.48±0.48	0.24±0.09	12.22±1.14	0.22±0.02	8.06±0.46	0.951
E. coli	0 (Untreated)	6.34±0.14	-2.76±0.13	0.27±0.03	9.24±0.32	-0.27±0.01	5.56±0.39	0.997
	4	5.28±0.14	-3.13±0.89	0.13±0.06	6.84±0.29	-0.15±0.03	-1.08±0.33	0.952
	6	5.18±0.92	-2.79±0.22	0.19±0.05	3.48±0.81	-0.19±0.03	-1.62±0.36	0.976
	8	4.72±0.14	-2.78±0.61	0.18±0.09	5.94±1.99	-0.18±0.01	0.34±0.49	0.930

A: decimal logarithm of initial load, log (CFU g^{-1}); **C**: difference in value of the upper and the lower asymptotes, log (CFU g^{-1}); ***B***: relative growth or death rate at time equal to M log (CFU g^{-1}) day^{-1}; ***M***: time at which growth or death rate (***B***) are maxima (day); μ_{max}**:** maximum exponential growth or death rate, log (CFU g^{-1}) day^{-1}; λ: lag time (day). R^2: determination coefficient adjusted for degree or freedom. Values ± SD.

Table 3

Monitored variable	PL-fluence (J cm^{-2})	Storage time (day)					
		0	**4**	**8**	**12**	**16**	**20**
SSC	**0 (untreated)**	4.75 ^{aC}	4.83 ^{bB}	4.25 ^{bA}	4.67 ^{bB}	5.13 ^{cC}	5.08 ^{cC}
	4	4.25 ^{aA}	5.22 ^{bC}	4.33 ^{aA}	4.33 ^{aA}	4.92 ^{bC}	4.83 ^{bBC}
	6	4.25 ^{aA}	4.33 ^{aA}	4.92 ^{bB}	3.92 ^{aA}	4.83 ^{bA}	4.50 ^{aA}
	8	4.50 ^{aB}	4.83 ^{bB}	4.42 ^{aA}	4.25 ^{aA}	4.92 ^{bB}	4.75 ^{bB}
pH	**0 (untreated)**	4.59 ^{aA}	4.65 ^{aA}	4.63 ^{aA}	4.62 ^{aA}	4.71 ^{bB}	4.75 ^{bB}
	4	4.54 ^{aA}	4.67 ^{aA}	4.62 ^{aA}	4.66 ^{bA}	4.64 ^{bB}	4.60 ^{aA}
	6	4.45 ^{aA}	4.69 ^{bA}	4.59 ^{aA}	4.74 ^{bB}	4.62 ^{aB}	4.66 ^{aA}
	8	4.35 ^{aB}	4.71 ^{bB}	4.58 ^{aA}	4.81 ^{cC}	4.46 ^{aA}	4.62 ^{aA}
TA	**0 (untreated)**	0.31 ^{aB}	0.29 ^{aAB}	0.20 ^{aA}	0.34 ^{cB}	0.29 ^{b}	0.25 ^{aA}
	4	0.39 ^{aB}	0.29 ^{aA}	0.29 ^{bA}	0.29 ^{bA}	0.31 ^{bAB}	0.33 ^{bB}
	6	0.34 ^{aC}	0.28 ^{aB}	0.29 ^{aB}	0.26 ^{aA}	0.25 ^{aA}	0.27 ^{aA}
	8	0.33 ^{aBC}	0.34 ^{bC}	0.29 ^{aB}	0.30 ^{bcB}	0.35 ^{cC}	0.27 ^{aA}

Values are the mean of three independent determinations of two replicates (n=6).

Different lower case letter in the same column denotes significant differences among treatments (p<0.05).

Different capital letters in the same row for each treatment denotes significant differences with cold storage time (p<0.05).

FIGURE CAPTIONS

Figure 1. Changes on the oxygen (□) and carbon dioxide (O) concentrations in the headspace of trays with tomato slices just after their exposure to PL-treatments. Points are the means of three repetitions from two replicate packages ±SD. Lines are the fit of second-order polynomials to the experimental data.

Figure 2. Effects of pulsed light treatments (PL) on the oxygen concentration in the headspace of trays containing fresh-cut tomatoes stored at 4 °C. Points are the mean of three repetitions from two replicate packages ±SD. Lines are the fit to the Gompertz's modified model to the experimental data. PL-treatments: Untreated (◆); 4 J cm^2 (□); 6 J cm^2 (∆) and 8 J cm^2 (O).

Figure 3. Effect of pulsed light treatments (PL) on the carbon dioxide concentration in the headspace of trays cointaining fresh-cut tomatoes stored at 4 °C. Points are the mean of tree repetitions from two replicate packages ±SD. Lines are the fit of the Gompertz's modified model to the experimental data. PL-treatments: Untreated (◆); 4 J cm^2 (□); 6 J cm^2 (∆) and 8 J cm^2 (O).

Figure 4. Fits of exponential decaying models to the counts of *Listeria innocua* (□) and *Escherichia coli* (O) in fresh-cut tomatoes packaged in plastic trays and exposed to pulsed light treatments (PL).

Figure 5. Changes in the *Listeria innocua* counts in fresh-cut tomatoes exposed to pulsed light (PL) and then stored at 4 °C for 20 days. Points are the means of 6 determinations ±SD. Lines are the fits of a modified Gompertz's model to the experimental data. PL-treatments: Untreated (◆); 4 J cm^2 (□); 6 J cm^2 (∆) and 8 J cm^2 (O).

Figure 6. Changes in the *Escherichia coli* counts on fresh-cut tomatoes exposed to pulsed light (PL) and then stored at 4 °C for 20 days. Points are the means of 6 determinations ±SD. Lines are the fits of a modified Gompertz's model to the experimental data. PL-treatments: Untreated (◆); 4 J cm^2 (□); 6 J cm^2 (∆) and 8 J cm^2 (O).

Figure 1

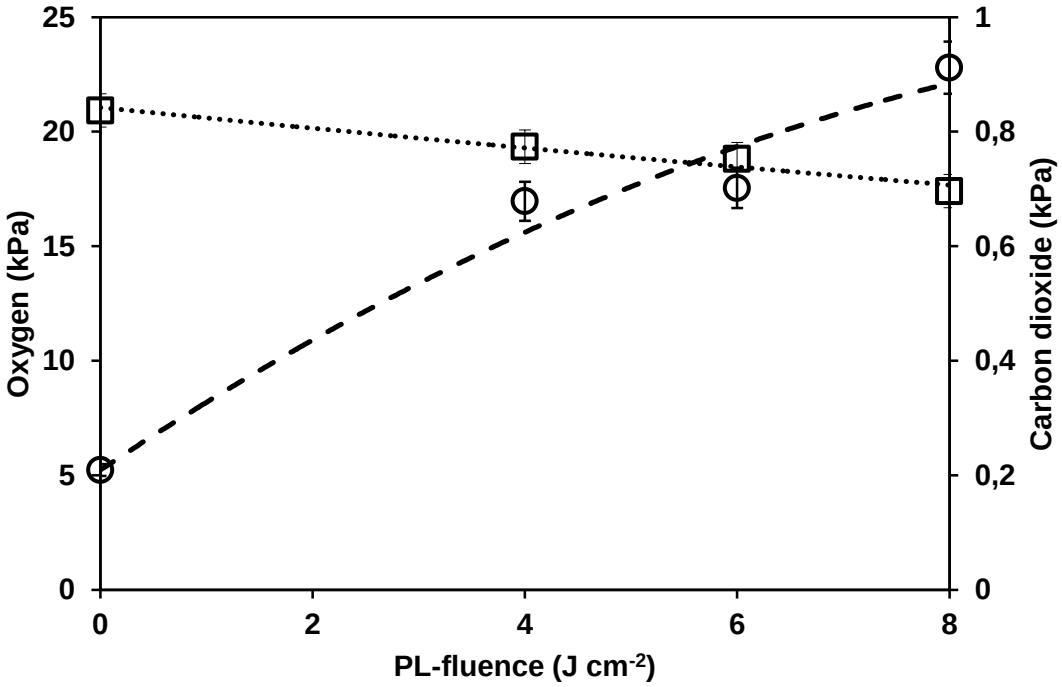

25
20
15
10
5
0
Oxygen (kPa)
1
0,8
0,6
0,4
0,2
0
Carbon dioxide (kPa)
0
2
4
6
8
PL-fluence (J cm-2)

Figure 2

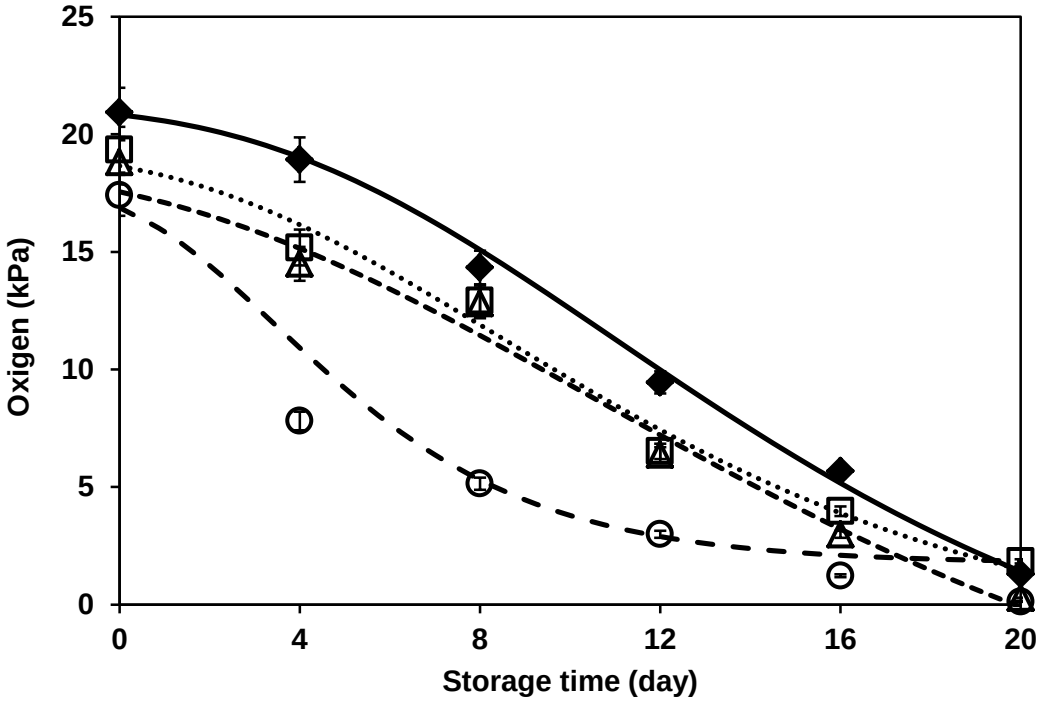


Figure 3

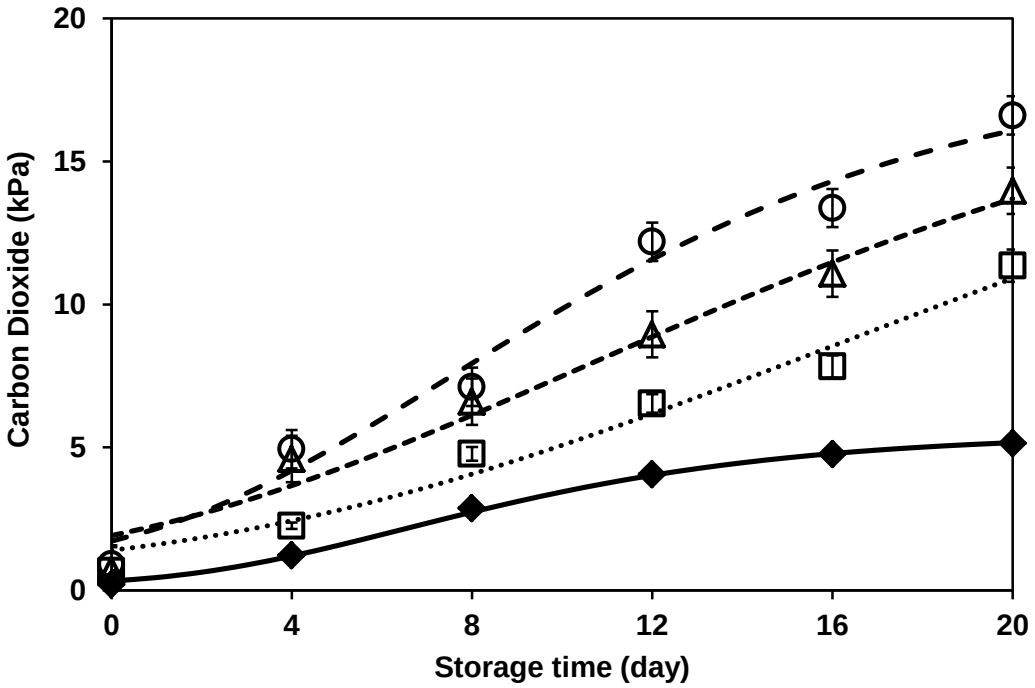


Figure 4

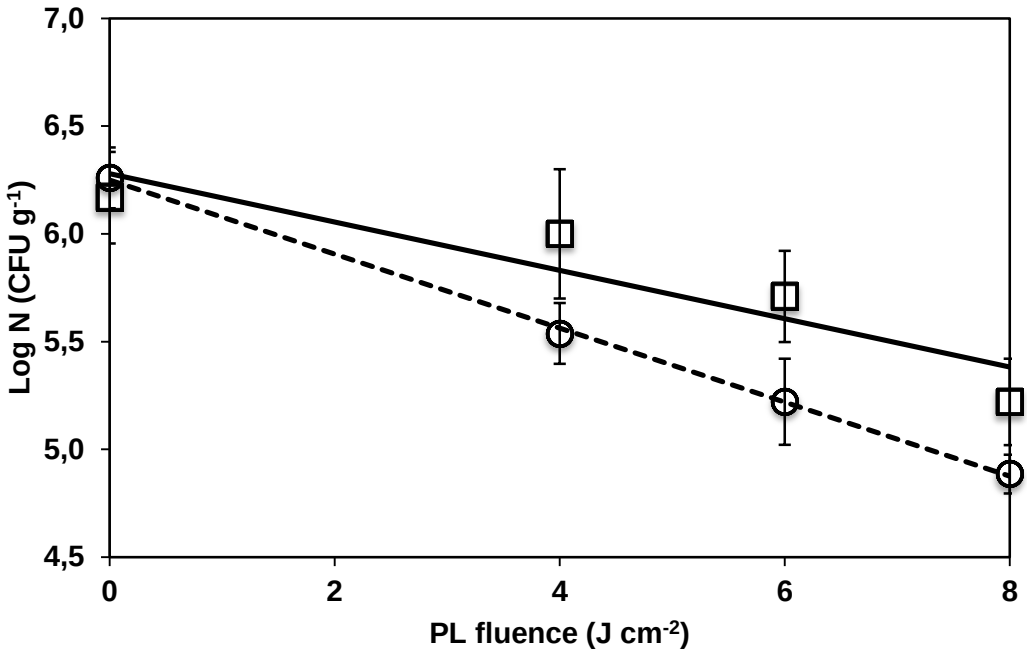


Figure 5

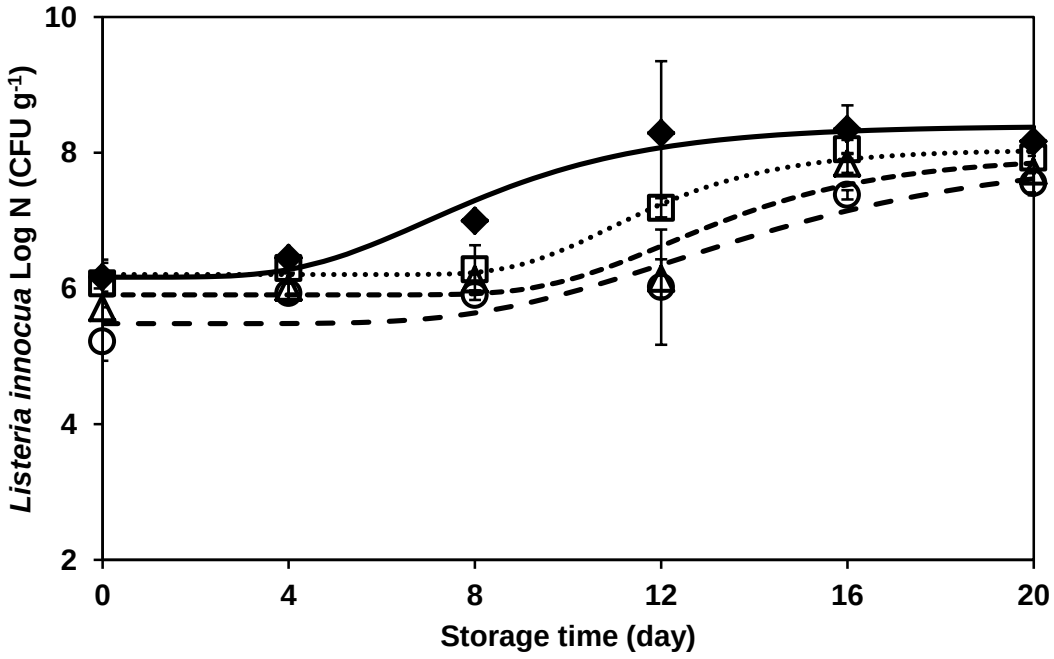
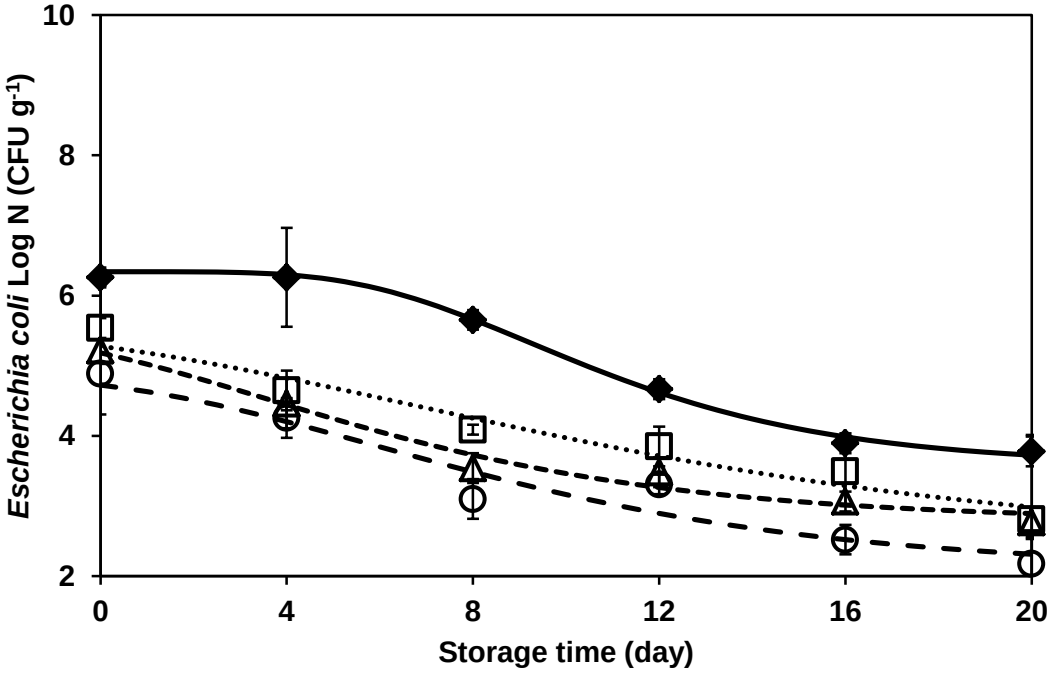

10
8
6
4
2
Listeria innocua Log N (CFU g-1)
0
4
8
12
16
20
Storage time (day)

Figure 6


10
8
6
4
2
Escherichia coli Log N (CFU g-1)
0
4
8
12
16
20
Storage time (day)